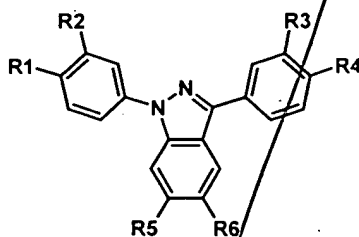


Claims:

1. Compounds represented by formula (I)



(Formula (I))

wherein

one of the radicals  $R^1$  or  $R^2$  and one of the radicals  $R^3$  or  $R^4$  is hydrogen and the other is independently  $-\text{COOH}$ ,  $-\text{COOR}^7$ ,  $-\text{CONH}_2$ ,  $-\text{CONH}(\text{CH}_2)_n\text{OH}$ , wherein  $n = 2 - 8$ ,  $-\text{CONR}^8\text{R}^9$ ,  $-\text{CH}_2\text{OH}$ ,  $-\text{CH}_2\text{NH}_2$ ,  $-\text{NO}_2$ ,  $\text{NR}^{10}\text{R}^{11}$ ,  $\text{NHCOR}^{12}$ ,  $\text{Cl}$ ,  $\text{Br}$ ,  $\text{F}$ ,  $-\text{CF}_3$ ,  $\text{O}(\text{C}_1-\text{C}_4)\text{-alkyl}$ , which could be substituted by methyl or phenyl at any of the carbons  $\text{C}_1-\text{C}_4$ ,  $-\text{N}=\text{C}=\text{O}$ ,  $\text{N}=\text{C}=\text{S}$ ,  $-\text{SO}_3\text{H}$ ,  $-\text{SO}_2\text{NH}(\text{CH}_2)_n\text{NH}_2$ ,  $(\text{C}_1-\text{C}_4)$  alkyl,  $(\text{C}_1-\text{C}_{16})\text{-alkyl}$  substituted at the terminal carbon with  $-\text{COOH}$ ,  $-\text{COOR}^7$ ,  $-\text{CONH}_2$ ,  $-\text{CONR}^8\text{R}^9$ ,  $-\text{CONH}(\text{CH}_2)_n\text{OH}$ , wherein  $n = 2 - 8$ ,  $-\text{CH}_2\text{OH}$ ,  $-\text{CH}_2\text{NH}_2$ ,  $-\text{N}=\text{C}=\text{O}$ ,  $\text{N}=\text{C}=\text{S}$ ,  $-\text{SO}_3\text{H}$ ,  $-\text{SO}_2\text{NH}(\text{CH}_2)_n\text{NH}_2$ ,  $-\text{CONH}(\text{CH}_2)_n\text{NH}_2$ , wherein  $n = 2 - 8$ , and the  $\text{NH}_2$ -group could also be substituted by  $(\text{C}_1-\text{C}_4)$  alkyl or a commonly used amino protecting group such as *tert*-butyloxycarbonyl, 9-fluorenylmethoxycarbonyl, phthalimido, trifluoroacetamido, methoxycarbonyl, ethoxycarbonyl, benzyloxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-(trimethylsilyl)ethoxycarbonyl,

and one of the radicals  $R^5$  or  $R^6$  is hydrogen and the other is hydrogen, halogen,  $\text{O}(\text{C}_1-\text{C}_4)\text{-alkyl}$  which could be substituted by methyl or phenyl at any of the carbons  $\text{C}_1-\text{C}_4$ ,  $-\text{NO}_2$ ,  $\text{NR}^{10}\text{R}^{11}$ ,  $\text{NHCOR}^{12}$ ,  $(\text{C}_1-\text{C}_4)$  alkyl,

(C<sub>1</sub>-C<sub>16</sub>)-alkyl substituted at the terminal carbon with -COOH, -COOR<sup>7</sup>, -CONH<sub>2</sub>, -CONR<sup>8</sup>R<sup>9</sup>, -CONH(CH<sub>2</sub>)<sub>n</sub>OH, wherein n = 2 - 8, -CH<sub>2</sub>OH, -CH<sub>2</sub>NH<sub>2</sub>, -N=C=O, N=C=S, -SO<sub>3</sub>H, -SO<sub>2</sub>NH(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub>, -CONH(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub>, wherein n = 2 - 8, and the NH<sub>2</sub>-group could also be substituted by (C<sub>1</sub>-C<sub>4</sub>) alkyl or a commonly used amino protecting group,

R<sup>7</sup> is a commonly used carboxyl protecting or carboxyl activating group

R<sup>8</sup> or R<sup>9</sup> is hydrogen and the other is lower alkyl (C<sub>1</sub>-C<sub>4</sub>), phenyl, benzyl, or R<sup>8</sup> and R<sup>9</sup> are part of a 5 or 6 membered ring.

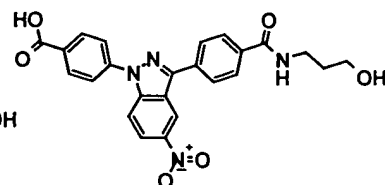
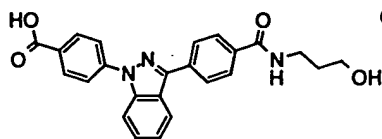
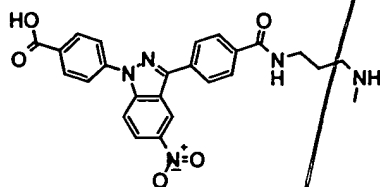
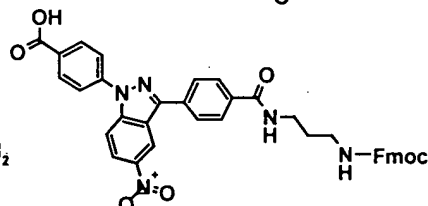
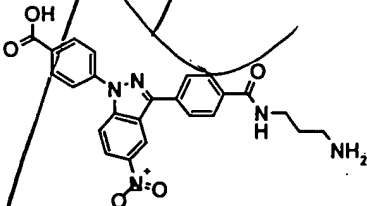
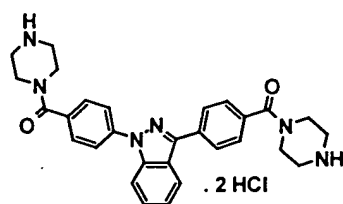
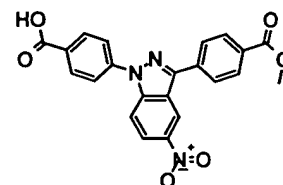
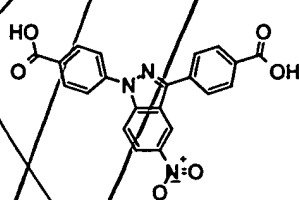
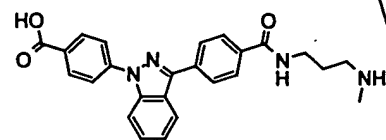
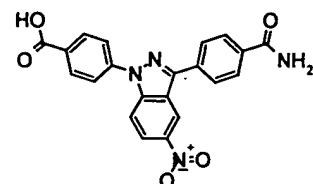
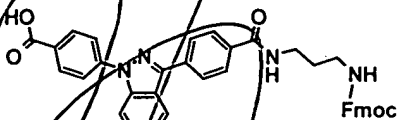
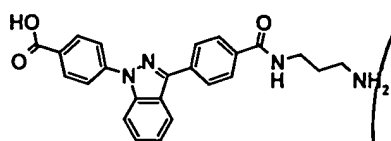
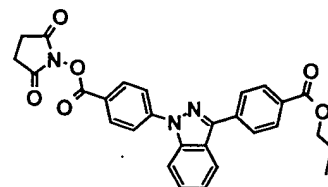
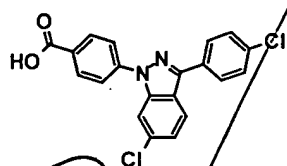
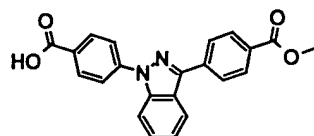
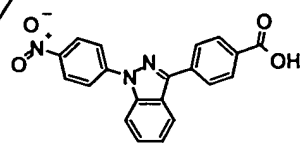
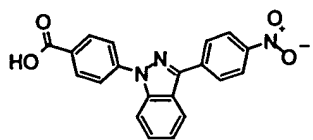
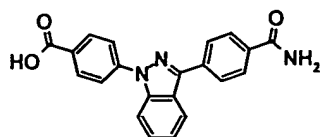
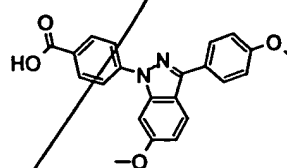
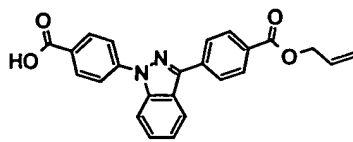
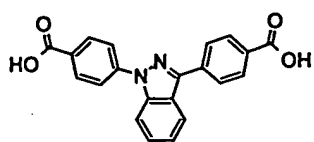
R<sup>10</sup> and R<sup>11</sup> are independently hydrogen or (C<sub>1</sub>-C<sub>4</sub>)alkyl

R<sup>12</sup> is (C<sub>1</sub>-C<sub>10</sub>)alkyl, phenyl, which both can be substituted by (C<sub>1</sub>-C<sub>4</sub>) alkyl, protected amino group or halogen.

2. Compounds according to claim 1 represented by the following structures:

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10507-33615260



3. Compounds represented by formula (II – III)

A-B-D-C-D'-E (Formula (II))

A-B-D-E-D'-C (Formula (III))

wherein

A is a solid support selected from standard materials applied in solid phase and solution phase organic chemistry.

B is a linker allowing cleavage of fluorescent conjugates of formula (II-III) for liberation of the D-C-D'-E or D-E-D'-C fragment, respectively.

C is a compound selected from formula (I)

D and D' are independently a bond or a spacer selected from  $\alpha,\omega$ -diamino-alkanes, diaminocyclohexyl, bis-(aminomethyl)-substituted phenyl,  $\alpha$ -amino- $\omega$ -hydroxy-alkanes, alkylamines, cyclic alkylamines or cyclic alkylidiamines or amino acids without or with additional functionality in the side chain.

E is the molecule to be investigated.

4. Compounds according to claim 3 wherein

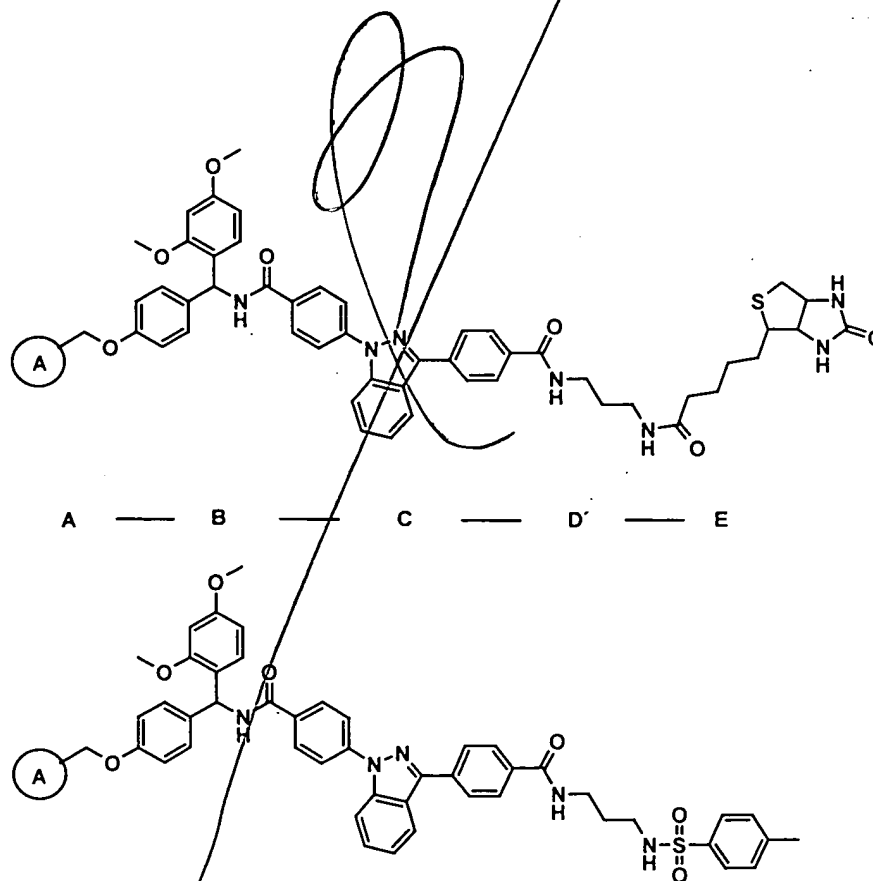
A is selected from functionalized polystyrene based resins, polyacrylamide based polymers, polystyrene / polydimethylacrylamide composites, PEGA resins, polystyrene-polyoxyethylene based supports, Tentagel, PEG-polystyrene graft polymeric supports, glass surfaces, functionalized surfaces, materials grafted with functionalized surfaces, or polyethylenglycol.

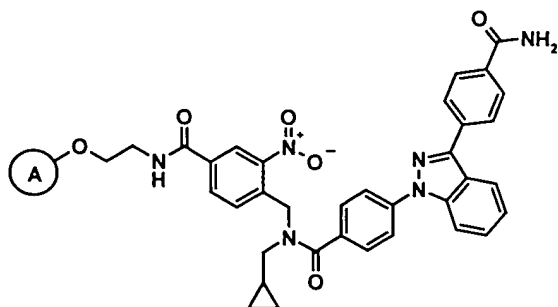
B is selected from benzyl, benzhydryl, benzhydryliden, trityl, xanthenyl, benzoin, silicon, or allyl based linkers.

C is a compound selected from formula (I)

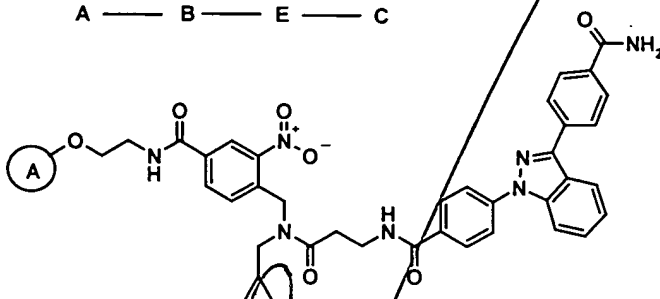
E is a low molecular weight compound, a peptide, a protein, a carbohydrate, a nucleic acid, or a lipid containing a functional group for conjugate formation

5. Compounds according to claim 3 represented by the following structures:





A — B — E — C



A — B — E — D' — C  
A — B — D — E — C

6. Compounds represented by formula (IV):

E-D'-C (Formula (IV))

wherein

E is the molecule to be investigated

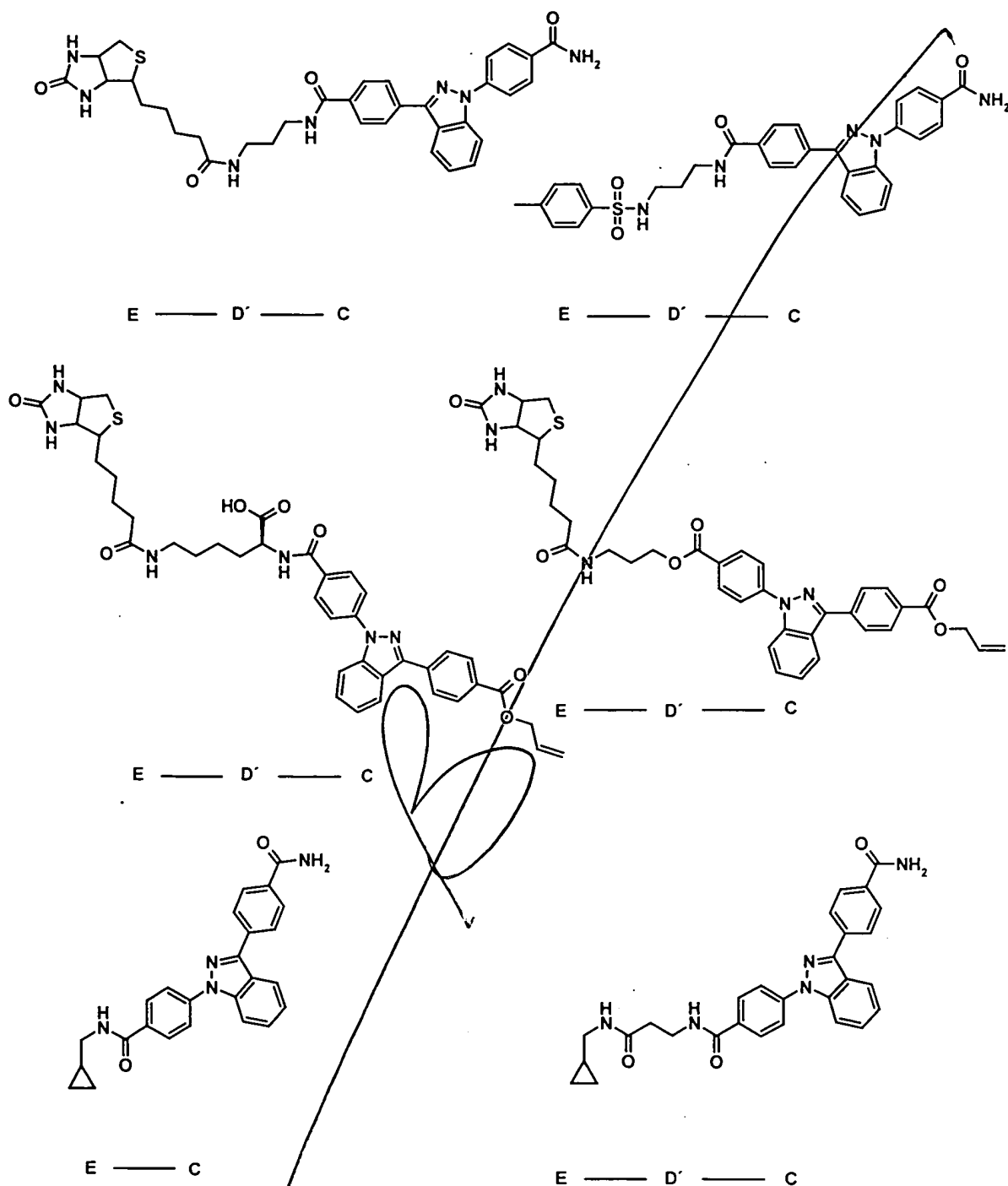
D' is a bond or a spacer selected from  $\alpha,\omega$ -diamino-alkanes, diaminocyclohexyl, bis-(aminomethyl)-substituted phenyl,  $\alpha$ -amino- $\omega$ -hydroxy-alkanes, alkylamines, cyclic

alkylamines, cyclic alkyldiamines or amino acids without or with additional functionality in the side chain

C is a compound selected from formula (I)

7. Compounds according to claim 6 represented by the following structures:

105070-6545250



8. A method for identification of an interaction between an AIDA labelled molecule and a binding molecule in homogeneous solution wherein the method comprises the following steps:



Step 1A: Providing an AIDA labelled molecule selected from formula (IV)

Step 1B: Admixing the AIDA-labelled molecule of formula (IV) with a binding molecule; and then

Step 1C: selectively detecting a binding event with the AIDA-labelled molecule described in Step 1B and the binding molecule by methods of fluorescence spectroscopy.

9. Method according to claim 8 wherein the methods of fluorescence spectroscopy are measurements of

- Increase of fluorescence anisotropy/polarisation of AIDA emission in continuous wave = prompt = steady state fluorometers,
- Increase of rotational correlation time in time-resolved fluorescence equipments
- Increase in translation diffusion time in single molecule fluorescence experiments determined from autocorrelation calculations on the time trace of fluorescence fluctuations,
- Increase or decrease of AIDA fluorescence emission in the wavelength range between 350 and 700 nm with excitation wavelengths in the range between 300 and 400 nm,
- Fluorescence resonance energy transfer (donor quenching or acceptor sensitisation) from excited tryptophan (donor) in the binding molecule which in this case is a peptide or protein to the AIDA dye (acceptor) in the molecule of the conjugate,
- Fluorescence resonance energy transfer (donor quenching or acceptor sensitisation) from the excited AIDA dye in the conjugate molecule (donor) to a fluorescent label (acceptor) of the binding molecule which in this case can comprise any compound class.

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10. A method for identification of an interaction between an AIDA labelled molecule on the solid support which is conventionally used in solid phase organic chemistry and a binding molecule in homogeneous solution containing the solid support wherein the method comprises the following steps:

Step 2A: Providing an AIDA labelled molecule as conjugate of formula (II or III)

Step 2B: Admixing the AIDA-labelled molecule as conjugate of formula (II or III) with a binding molecule; and then

Step 2C: selectively detecting a binding event with the AIDA-labelled molecule described in Step 2B and the binding molecule by methods used in fluorescence spectroscopy resulting in a quantitative signal providing a means to identify the AIDA-linked molecule with the highest binding affinity to the binding molecule,

Step 2D: Isolation of the solid support containing the identified AIDA-molecule represented by formula (II or III)

Step 2E: : Selectively detecting a binding event with the AIDA-labelled molecule described in Step 2D and the binding molecule by various methods used in fluorescence spectroscopy described in the procedure 1A-C.

11. Method according to claim 10 wherein the fluorescence spectroscopic methods in step 2C are

- Direct detection of binding of fluorescently labelled macromolecules to AIDA containing solid supports applying confocal microscopic and spectroscopic techniques
- measurement of enhancement of the change in molecular brightness by chemically linking AIDA to a second environmentally sensitive molecule as

commonly used in conventional fluorescence spectroscopy performed during the synthesis of the compound on the solid support,

- measurement of fluorescence resonance energy transfer: From AIDA to a suitable long wavelength dye which will thereby be sensitised using AIDA UV-excitation detected by change in molecular brightness at the emission wavelength of the long wavelength dye,
- measurement of fluorescence resonance energy transfer: Reduction of specific brightness of AIDA on the molecule linked to the solid support at 351nm excitation and 400 nm emission wavelengths,
- Detection of the change in quantum yield by measuring reduction or increase in molecular brightness by time-resolved single molecule spectroscopy.

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